

Photoreceptors and visual pigments in the red-eared turtle, *Trachemys scripta elegans*

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Abstract

Absorbance spectra of cone outer segments and oil droplets were recorded microspectrophotometrically in the retina of the red-eared turtle, *Trachemys scripta elegans*. There are four cone visual pigments, with λ_{\max} = 617 nm (red sensitive), 515 nm (green sensitive), 458 nm (blue sensitive), and 372 nm (UV-sensitive). The red-sensitive pigment resides in single cones with red or orange oil droplets, and in both members of double cones. The principal member of the double cone contains an orange oil droplet, and the accessory member is droplet free. The green-sensitive pigment is situated in single cones with orange/dark yellow droplets. The blue-sensitive pigment is combined with the UV-absorbing oil droplet in single cones. The UV-sensitive pigment resides in single cones with clear oil droplets that exhibited virtually no absorbance down to 325 nm. Thus, seven types of cones can be identified based on their morphology, oil droplet color, and the visual pigment absorbance. At the moment, this is the most complex cone system described for vertebrates.

Keywords: Retinal cones, Visual pigments, Oil droplets, Turtle, Microspectrophotometry

Introduction

In turtle retinas, up to six types of cones were identified on the basis of morphological difference, color of the oil droplet, and the visual pigment residing in the outer segment (Liebman & Granda, 1971; Liebman, 1972; Kolb & Jones, 1982, 1987; Lipetz & MacNichol, 1982; Ohtsuka, 1984, 1985a, 1985b; Zueva, 1982). In the best-studied red-eared turtle, *Trachemys scripta elegans*,* the presence of double cones and four types of single cones is reported. Double cones consist of a longer oil droplet-containing principal member, and a shorter and thicker accessory member that lacks an oil droplet. All single cones contain oil droplets, and can be subdivided into two types of large single cones, and two types of smaller cones (Kolb & Jones, 1982, 1987). Three cone visual pigments, absorbing maximally (in freshwater turtles) around 620 nm (further called red sensitive), 530 nm (green sensitive), and 460 nm (blue sensitive) were found by electrophysiological spectral-sensitivity measurements (Baylor & Hodgkin, 1973; Ohtsuka, 1984, 1985a, 1985b) and microspectrophotometry (MSP) (Liebman & Granda, 1971; Liebman, 1972; Lipetz & MacNichol,

1982). Oil droplets of red, orange, yellow, and pale green colors, as well as colorless droplets, were detected within the same retina.

However, there is much controversy in the attribution of specific visual pigments and oil droplet colors to particular morphological types of cones. In their pioneering MSP study, Liebman and Granda (1971) and Liebman (1972) placed the *Pseudemys* red-sensitive pigment in one member of double cones (oil droplet color not reported, probably orange) and in single cones with red droplets. Green-sensitive pigment was found in the accessory member of the double cone, and in single cones with orange droplets. Blue-sensitive pigment resides in single cones with colorless droplets. In the most detailed electrophysiological study to date, Ohtsuka (1984, 1985a) found the accessory cone, like the principal cone, to be red sensitive. In addition, the red-sensitive pigment is placed in single cones with red and pale green droplets. A green-sensitive pigment was found in a class of single cones having orange droplets, and the blue-sensitive pigment in small single cones having colorless droplet. The same distribution of visual pigments/oil droplets was also reported for *Geoclemys* (Ohtsuka, 1984, 1985b). The most extensive MSP study of turtles was performed by Lipetz and MacNichol (1982) on *Chrysemys scripta elegans*. They reported a red-sensitive pigment in both members of the double cones, two types of red-sensitive single cones (having red and colorless droplets), and a green-sensitive pigment in single cones having orange droplets. A short wavelength-sensitive pigment was found in small single cones having clear droplets with an absorbance peak in the violet. This corresponds to the pale green droplets reported by Ohtsuka (1984, 1985a), previously characterized by Fujimoto et al. (1957).

*In the visual literature, the red-eared turtle is usually called *Pseudemys scripta elegans*, even after placement in the genus *Trachemys* in 1986. Sometimes even the older name *Chrysemys s.e.* is used. In this report, we shall use the names applied by the cited authors.

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Thus, there is a discrepancy in the visual pigment attributed to the accessory cone that is believed to be either green (Liebman & Granda, 1971) or red sensitive (Lipetz & MacNichol, 1982; Ohtsuka, 1984, 1985*a*). Single cones with pale-green droplets have been reported to contain either red (Ohtsuka, 1984, 1985*a*) or blue-sensitive (Lipetz & MacNichol, 1982) pigment. Cones with absolutely colorless droplets are either blue (Ohtsuka, 1984, 1985*a*) or red sensitive (Lipetz & MacNichol, 1982). In addition, the presence of UV-sensitive cones was inferred based on cone morphology (Kolb & Jones, 1987; Goede & Kolb, 1994; Amermüller & Kolb, 1996) and on recordings from horizontal cells (Amermüller et al., 1998), but have not been detected using MSP.

The importance of establishing a connection between the morphological type of a photoreceptor and its oil droplet and visual pigment, for understanding the design of the color vision system in reptiles and birds, made us re-investigate the problem. Here we show that the retina of the red-eared turtle contains four cone visual pigments, red, green, blue, and UV sensitive, forming ordered combinations with oil droplets and residing in seven subtypes of cones. Our attribution of visual pigments to oil droplet types differs from that of Lipetz and MacNichol (1982) and Ohtsuka (1984, 1985*a*).

Methods

Young (approximately 13-cm carapace length) red-eared turtles, *Trachemys scripta elegans*, were obtained from Glades Herp, Inc., Fort Myers, FL, and kept in a terrarium with access to water for 2 weeks prior the experiments. Three animals were dark adapted overnight, narcotized with an injection of ketamine, and decapitated. Retinas were isolated under infrared using an appropriate image converter, and MSP performed as previously described (Loew et al., 1996). Measurements were made on photoreceptors protruding from the retinal edge, where the morphological class of the cell could be determined with certainty. Both the outer segment and, when present, the oil droplet absorbance were recorded from

each cell. The measurements were made mostly from the central retinal area. No attempt was made to map the retinal distribution of different cone classes and we can only state that all photoreceptor types described were always present within the same neighborhood.

The λ_{\max} of the visual pigments was estimated by best fitting an A_2 -template based on carp porphyropsin using a modified Mansfield-MacNichol transform (Govardovskii et al., 2000). Fitting was only done on good quality spectra that exhibited a clear α -peak without obvious distortion and had a horizontal stretch of low absorbance at the long-wave end. The data are presented as the mean \pm one S.E.M.

Results

A sparse rod population containing a visual pigment with $\lambda_{\max} = 518 \pm 0.7$ nm was found, which, judging from the spectrum shape and the peak of the bleaching product at 400 nm, is A_2 -based (average of 14 spectra; data not shown). Four cone visual pigments were found: a long-wavelength red (LWS) with $\lambda_{\max} = 617 \pm 1.3$ nm (14 spectra), middle-wavelength green (MWS) with $\lambda_{\max} = 515 \pm 0.8$ nm (25 spectra), short-wavelength blue (SWS) with $\lambda_{\max} = 458 \pm 2.6$ nm (11 spectra), and UV spectra (UVS) with $\lambda_{\max} = 372 \pm 1.2$ nm (12 spectra) (Fig. 1). The 3 nm difference in λ_{\max} between the visual pigments of rods and MWS cones, while small, is real ($P > 0.97$, t -test). Actually, the entire averaged curve for the rod pigment is shifted toward longer wavelength compared to the green cone pigment (data not shown).

For counting various visual pigment/oil droplet combinations, we used all cells that allowed unambiguous identification of their spectral type. Two from a total of 142 cones could not be classified based on this criterion. The LWS pigment was found in long single cones with red (33 cells) or orange (13 cells) oil droplets, and in both members of double cones (14 pairs) (Fig. 2A). The principal member of the double cone contained an orange oil droplet, while the accessory member was droplet free. The MWS pigment was found in long single cones with yellow droplets (40 cells). The

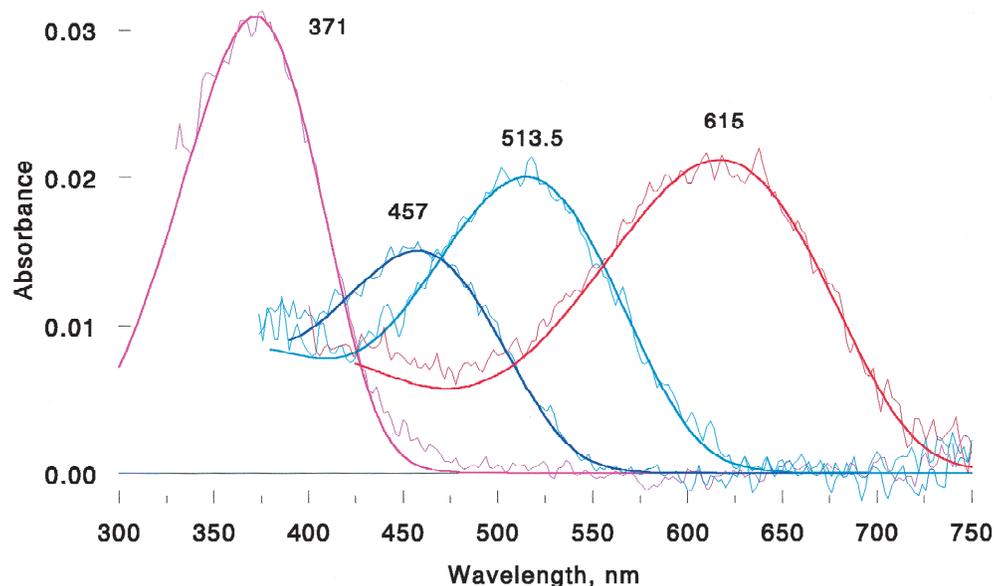


Fig. 1. Absorbance spectra of four types of cone visual pigments in the *Trachemys* retina. Each thin noisy curve is an average of recordings from 10 to 15 cone outer segments. Smooth thick lines are corresponding porphyropsin templates.

SWS pigment was combined with the UV-absorbing, colorless oil droplet in small single cones (21 cells). The UVS pigment resided in small single cones with clear, colorless oil droplets (21 cells) that exhibited virtually no absorbance down to 325 nm (Fig. 2A).

Since previous studies had associated the colorless oil droplets with blue or red absorbing visual pigments and not a UVS one, the possibility that the UV peak reported here could, in fact, represent a photoproduct of a more long-wave absorbing pigment should be ruled out. The evidence for the presence of an independent UV-absorbing visual pigment rather than a photoproduct is similar to those used in previous studies on UV pigments (Hárosi, 1985; Loew, 1994; Loew et al., 1996):

1. The absorbance peak at 372 nm is bleachable, that is, its height decreases after illumination.
2. The peak is dichroic, with maximum absorption of light when the electric vector is orientated parallel to the disk's plane, as expected for visual pigments (dichroic ratio 1.7 ± 0.5 , 4 cells).
3. The absorbing dipole of the postbleach product is orientated predominantly perpendicular to the disk's plane, again as seen for other cone pigments.
4. The alpha-band maximum in the UV cones is markedly higher than the alpha-peak of long-wave cones (Fig. 1A), while the postbleach absorbance of the long-wave cone pigments never exceeds the level of the beta-band, ca. 0.4 alpha maximum (data not shown).

Exact characterization of the optical properties of oil droplets is a hard task. A substantial fraction of the MSP measuring light leaks around these small, highly absorbing structures due to scatter and diffraction. Thus, directly measured absorbance rarely exceeds 1.0 (Lipetz, 1984), and the shape of the droplet spectra at optical density (OD) > 0.8 carries little information about actual light absorption. This should be kept in mind when evaluating the absorbance spectra of oil droplets seen in Fig. 2A. However, useful classification can be achieved by using the cut-off wavelength that is defined as the wavelength of 50% transmittance/absorbance ($\lambda_{0.5}$) since it is determined from a part of the curve that is relatively insensitive to the light leakage error. Cone oil droplets in the *Trachemys* retina can be subdivided into six classes (Fig. 2A).

Cut-off wavelengths of the brightly colored droplets are shown in Fig. 2B. Red droplets with $\lambda_{0.5} = 595$ nm are associated with the LWS single cones. The classification of orange/yellow droplets based solely on their visible color is unreliable because their absorbance spectra are rather similar. Two types of droplets with longer cutoffs are called orange. Larger droplets with slightly longer $\lambda_{0.5}$ are found in principal members of double cones. Smaller droplets with shorter $\lambda_{0.5}$ belong to a specific population of red-sensitive single cones that has not been revealed earlier. The separation into two classes on the basis of the cell of origin corresponds to the separation on the basis of maximum optical density, which probably originates from the difference in the droplets' diameter, and, hence, different percentages of leaked light. The dotted lines at the top of the RS2 curve in Fig. 2A shows \pm one S.E.M. limits of data for smaller droplets from single cones; approximately the same scatter is observed in larger and more dense droplets from the principal members of double cones. The shortest-wave droplets of this group, called yellow, have the largest diameter and highest OD, and are associated with MWS single cones. Accessory members of double cones lack oil droplet but contain a dispersed yellow substance in their inner segment. Its absorbance spectrum peaks at ca. 450 nm and does not exceed 0.2 OD. Similar oil droplet complement has previously been reported by Zueva (1982) in two other species of turtles, dry land-living *Testudo horsfieldi* and swamp-living *Emys orbicularis*, though the corresponding visual pigments have not been identified.

We also recorded a few visual pigment/oil droplet combinations (all in single cones) that do not fit the above-described scheme. These were a P617₂ + pale green droplet (1 cell), P516₂ + clear droplet (1), P458₂ + orange droplet (1), P458₂ without droplet (1), and P372₂ without a droplet (1). The last two cells were probably not the accessory members of dissociated double cones since there was no principal member nearby and the cell shape resembled standard single cones rather than fat, short accessory members. These aberrant cells were found near standard photoreceptors of all types.

Data from different sources on photoreceptor types in the *Trachemys* retina are summarized in Table 1. It is seen that the tentative association of the MWS pigment with the accessory member of double cones by Liebman and Granda (1971) finds no support in later works. The discrepancy between our MSP data and the electrophysiological data of Ohtsuka (1984, 1985a) that asso-

Table 1. Morphological types of photoreceptors, visual pigments and oil droplets in the *Trachemys* retina^a

Source	Cell type							
	Rods	Principal	Accessory	Single 1	Single 2	Single 3	Single 4	Single 5
(1)	518	620? O?	518 —	620 R	620 O?	518 O	450 C/N?	
(2)	520	620 Y 495	620 —	620 R 572	—	540 O 530	620 PG 413	450 C
(3)	519	623 O 560	623 —	623 R 580	—	522 Y 525	462 C	622 N
Present study	518	617 O 552	617 —	617 R 595	617 O 548	515 Y 533	458 C 413	372 N

^a(1) Liebman & Granda (1971); Liebman (1972); (2) Ohtsuka (1984, 1985a); and (3) Lipetz & MacNichol (1982). Oil droplets are designated accordingly to the color reported by authors (R = red, O = orange, Y = yellow, PG = pale green = C = clear with the UV-absorbing peak, N = no selective absorbance), and by the wavelength of 50% transmittance, when available. $\lambda_{0.5}$ in (2) is for *Geoclemys reevesii*, from Fujimoto et al., 1957.

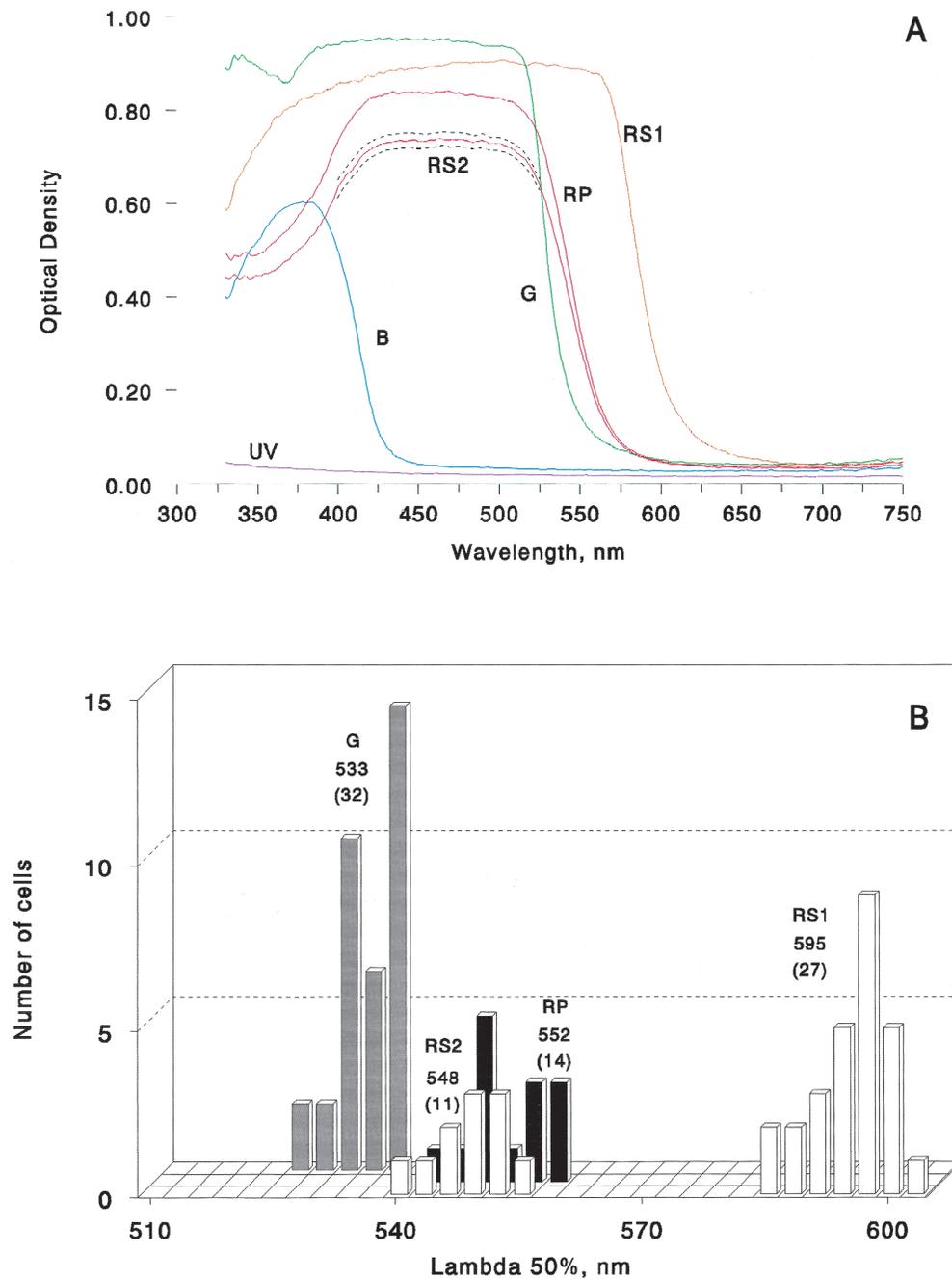


Fig. 2. Oil droplets from various types of cones. (A) Absorbance spectra. Each curve is an average of 10 to 15 droplets. RS1, RS2 are droplets from two types of red-sensitive single cones. RP: red-sensitive principal members of double cone; G: green-sensitive cones; B: blue-sensitive cones, and UV: ultraviolet-sensitive cones. Dotted lines at the top of the RS2 curve show \pm S.E.M. limits. (B) Frequency distribution of the wavelength of 50% transmittance of red, orange, and yellow droplets. Average $\lambda_{0.5}$ values and the number of cells recorded are shown near corresponding histogram clusters.

ciated LWS and SWS pigments with cones having pale green and completely colorless droplets is hard to explain. On the other hand, the UV-sensitive cones with transparent droplets detected by us well might have been overlooked in the previous MSP works. The recordings by Lipetz and MacNichol (1982) did not extend in the UV far enough to positively identify the pigment (E. F. MacNichol, Jr., personal communication). The cells with no clear absorbance in the visible region then could be considered as accidentally

bleached red-sensitive cones, as happened previously with the UV receptors in geckos (Loew, 1994; Loew et al., 1996). So the prediction of Kolb and Jones (1987), based on morphological evidence, and of Amermüller et al. (1998) from horizontal cell recordings, for the existence of UV-sensitive cones in *Trachemys* (their *Pseudemys*) is corroborated. However, we found it difficult to discriminate the UV-sensitive cells from other cones on the basis of their smaller size, as suggested by Kolb and Jones (1987), due

to a wide scatter of cell dimensions even within a restricted retinal area. Our last finding is an extra type of single red-sensitive cone with orange droplets. In previous studies, these cells were probably not separated from principal members of double cones bearing similar droplets and containing the same visual pigment.

Thus, accordingly to our data, the retina of the red-eared turtle contains seven types of cones that can be identified based on their morphology, oil droplet color, and the visual pigment absorbance. At the moment, it is the most complex cone system found in vertebrates.

Acknowledgments

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Note added in proof:

Coincident with the submission of this paper, we became aware of the work of Ventura et al. (reported in *Visual Neuroscience*, **19**, 759–765) who electrophysiologically derived the spectral sensitivity function for the UV cone in the turtle. Both our visual pigment data and their electrophysiological data peak at 372 nm confirming by two independent methods the λ_{med} of the UV cone. We thank Dr. Ventura for allowing us access to their data prior to its publication.

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